Systems Virology
of Emerging Respiratory Viruses

T003.0P Nanochip Bioanalyzer Protocol

(4/10/10 Lynn Law)

1. Turn on the bioanalyzer with the switch in the rear. The lamp on the front of the bioanalyzer will be solid green when ready. Only now, turn on the PC. Allow the bioanalyzer to warm up for a minimum of 30 minutes.
2. **Wear your gloves** to prevent electrostatic discharge and RNase contamination.
3. Remove reagents (Gel, dye, Nanomarker) from the fridge and equilibrate at room temp for 30 min (or water bath for a min). Protect from light.
4. Vortex dye and pulse.
5. Add 1 X of dye to a 65 µl aliquot of filtered gel.
6. Vortex, and spin at 13,000 x G for 10 min.
7. Prepare a 100ng/µl solution of each RNA sample to be analyzed.
8. Clean the electrode with RNAzap. Use the provided cleaning chip and do not exceed 350 µl or electrodes will short out during the run. Close lid & soak for 1min. Rinse several times with pure nuclease free water (in a separate cleaning chip). Repeat 5-10 times to remove any trace of RNAzap. Open lid, let dry 10 sec.
9. Denature ladder and samples in the 70C heat block for 2 min, chill on ice. Samples will remain denatured for over an hour, if kept on ice.
10. Work quickly through steps 11-20. For best resolution these steps should take less than 5 min.
11. Put chip on priming station. Pipette 9 µl of gel-dye mix into well G. (black dot)
12. Ensure plunger is at 1 ml, all the way up, close chip station, make sure it clicks.
13. Depress plunger until it locks in the clip.
14. Release clip at exactly 30 sec after locking the clip. Wait 5 sec, and then gently pull plunger all the way up.
15. Pipette 9 µl of gel dye mix into the 2 other wells marked “G”.
16. Pipette 5 µl of NanoMarker (buffer) into the ladder well and into each sample well.
17. Pipette 6 µl of nuclease free water into unused sample wells. A dry well blocks the current for the entire chip.
18. Load 1 µl of ladder or sample into the sample wells.
19. Vortex chip for 1 min. Start your run promptly.
20. Put the chip into the Bioanalyzer and gently close. Run the program. (Assay/eukaryotic total RNA/)
21. Immediately remove the chip when the run is over. Put the chip containing 350ul of fresh nuclease free diH2O into the machine and soak 10 sec to clean the electrodes. Leave the lid open for 10 sec to dry the electrodes.
22. Your file is automatically saved to the C drive of this computer. You can access it by date.

To access your data, open your file and click print. This will save your file rather than printing it. Browse to transfer and save your file.

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